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Benefits Of Probiotics Consumption In Adults With Allergic Rhinitis: A Meta-analysis

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ACCEPTANCE

This thesis, BENEFITS OF PROBIOTICS CONSUMPTION IN ADULTS WITH ALLERGIC RHINITIS: A META-ANALYSIS, by Bochuan Xie was prepared under the direction of the Master's Thesis Advisory Committee. It is accepted by the committee members in partial fulfillment of the requirements for the degree Master of Science in the Byrdine F. Lewis School of Nursing and Health Professions, Georgia State University. The Master's Thesis Advisory Committee, as representatives of the faculty, certify that this thesis has met all standards of excellence and scholarship as determined by the faculty.

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ABSTRACT

BENEFITS OF PROBIOTICS CONSUMPTION IN ADULTS WITH ALLERGIC RHINITIS: A META-ANALYSIS

by
Bochuan Xie

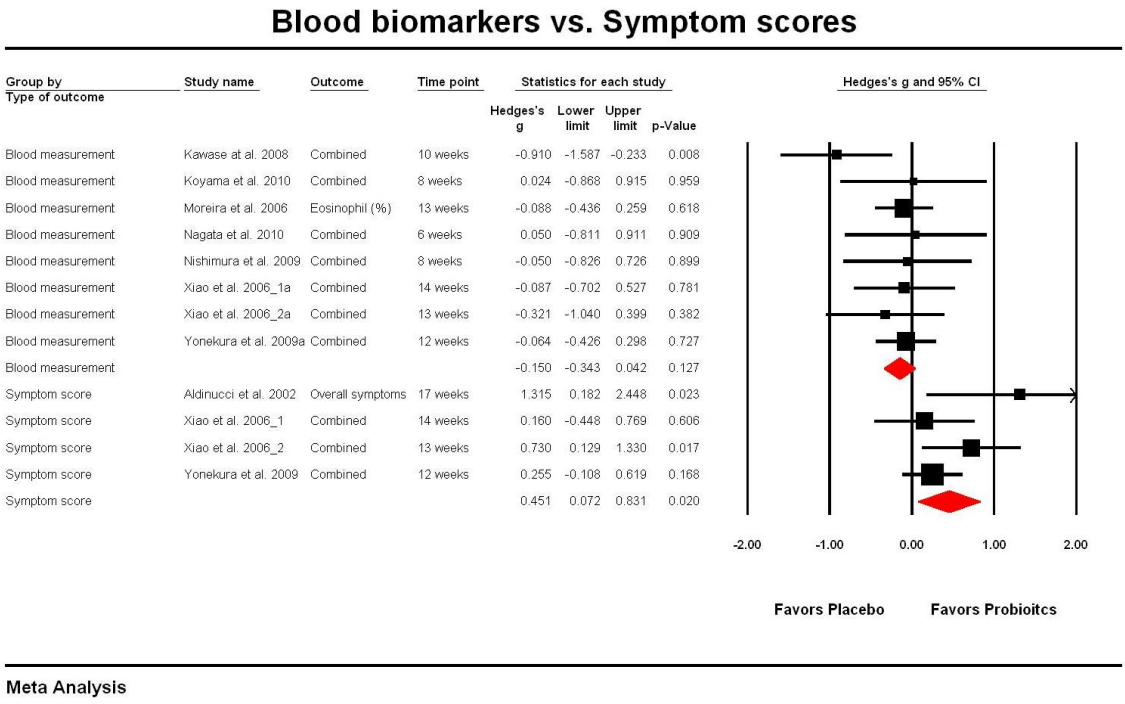
Background: About 1 in every 6 Americans suffer from seasonal allergies, aka allergic rhinitis (AR), every year. Typical symptoms of AR include sneezing, stuffy nose and watery eyes. Currently, two types of medications, anti-histamines and corticosteroids, are widely used for relieving AR symptoms; however, in addition to the concern about drug resistance after their long-term use, they also cause side effects such as dry mouth and dizziness.

Introduction: AR results from an abnormally heightened immune response triggered by unharmful substances in the environment. Therefore, a therapy capable of regulating the overall immune function of the body should alleviate the discomfort caused by AR. The intestine is the largest immune organ of the body. And consumption of probiotics can induce positive changes in the composition of the microbiota that reside in the intestine within a short period of time. As a result, several trials have been conducted to investigate the therapeutic effects of probiotics on AR. However, because of the generally small sample size of these studies, it is difficult to reach a definite conclusion. Therefore, we performed a meta-analysis to assess if probiotics consumption leads to improvement of AR symptoms.

Method: Key words of "probiotics" and "seasonal allergy" were used to search PubMed, Cochrane and CINAHL to identify randomized controlled trials for the meta-analysis. Out of the 69 initially identified papers, 9 were eventually included in the analysis. Studies excluded were either duplicates, reported un-relevant outcomes, or provided insufficient data for further analysis. The meta-analysis was conducted through CMA 2.0, commercial comprehensive meta-

analysis software. Standardized mean difference was calculated for subjective symptoms scores and serum biomarker levels for each study as the effect size, and the random effects model was applied to calculate the overall effect.

Results:



As shown in the Forest plot above, the Hedges' g value for overall symptom score was 0.451 with a p value of 0.02, indicating that probiotics consumption induced a moderate improvement in symptoms, and the improvement is statistically significant. On the other hand, the Hedges' g value for blood biomarkers level was -0.15 with a p value of 0.127, meaning probiotics performed worse than placebo at decreasing the inflammation at cellular or molecular level, as measured by serum biomarkers, but this inferior effect was not statistically significant.

Conclusion: The use of probiotics is beneficial at relieving allergic symptoms for AR patients, but contradictory findings were discovered when it comes to the measurement of serum biomarkers levels. Future studies are needed to identify reliable biomarkers for AR, and studies

with similar design but larger sample size would be helpful to further investigate the effectiveness of probiotics in the management of AR.

BENEFITS OF PROBIOTICS CONSUMPTION IN ADULTS WITH ALLERGIC RHINITIS: A META-ANALYSIS

by
Bochuan Xie

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Chapter I

Benefits of probiotics consumption in adults with allergic rhinitis: A Meta-Analysis

Introduction

The layman's term of allergic rhinitis (AR) is seasonal allergies, which describes the state of having various nasal and ocular symptoms. These symptoms occur as a result of the body's abnormally heightened immunological response to inhaled substances from the environment, such as tree or grass pollen, dandruff of cats or dogs, and dust mites. In addition to causing the physiological symptoms that are familiar to most people, e.g. itching, sneezing, runny nose, stuffy nose, watery eyes and headaches, AR is closely associated with significant emotional stress which negatively affects every aspect of a person's daily life, including school, work, exercise, as well as social life[1,2]. Based on a 2012 report, 10% to 20% of people living in industrialized countries suffer from AR every year[2]. In the U.S., the prevalence of AR is estimated to be 16%, translating to 40 million people[3]. What's worse is that, when comparing studies spanning the past decades, the prevalence of AR has been increasing globally[2].

In people who have AR, the uncomfortable and sometimes miserable symptoms arise because protective physiological functions are out of control. Under normal conditions, the nasal cavity adjusts inhaled air to a comfortable temperature and level of humidity before it reaches the lung. Such important functions are achieved thanks to the densely packed capillary network in the nose, which establishes a highly efficient interface for heat exchange, and the seromucous

glands located in the cavity that constantly secret mucus. Additionally, the mucus traps large airborne particles, such as dust and pathogens, thus functioning as the first line of defense.

However, when these homeostatic and defensive functions are impaired, which is the case in AR, characteristic allergic symptoms appear. Stuffy nose and subsequent headaches result from the blockage of the nasal airway caused by the abnormal engorgement of the blood vessels; runny nose is the direct effect of excessive mucus secretion[4].

The development of allergic responses in the nasal cavity consists of three stages: allergen sensitization, acute response, and chronic allergic inflammation[1,4]. During the first stage, antigen presenting cells (APCs) engulf allergens upon the encounter. The allergens are then broken down into peptide segments, which are later presented to naïve T cells (Th0 cells) once the APCs migrate into the lymph nodes. This interaction between APCs and Th0 cells leads to the multiplication and differentiation of Th0 cells, giving rise to either Th1 or Th2 cells. Th1 cells have been studied intensively because of their immune regulatory function. Th2 cells have the ability to bind with B cells, triggering their transformation into plasma cells that can produce and secret IgE into blood circulation. The high-affinity receptors on the cell membrane of mast cells and basophils enable their capturing of the circulating IgEs. This also signifies the completion of the stage of allergen sensitization. Whether the Th0 cells develop into Th1 cells or Th2 cells depends on the signals they receive from both the APCs and the immediate environment. Dendritic cells (DCs), the most thoroughly studied APCs by far, are found to play a crucial role in this transformational process. Once again, the precursor DCs could be activated to become either DC-1 cells or DC-2 cells, depending on the chemical signals in the vicinity. When Th0 cells interact with DC-1 cells in an IL-12 rich environment, Th1 cells are generated. In contrast, if the interaction taking place is between Th0 cells and DC-2 cells in an environment

with high level of IL-4, Th2 cells are formed. High levels of Th2 cell count have been reported to strongly relate to seasonal allergies. The second stage is acute response that initiates within minutes after the inhalation of a previously exposed allergen. The first step is the recognition of the allergens by the mast cells and basophils through the allergen-antibody (IgE in this case) interaction. The subsequent binding of multiple mast cells and basophils with each single allergen molecule leads to the cross linking between IgEs, thus opening the calcium channel of these cells. As a result, these cells are activated and begin to release the mediators that are packaged in the intracellular granules. Mediators involved at this step include histamine, proteases and leukotrienes. After reaching the intracellular space, each mediator binds to its specific receptors, which are located either on blood vessels or glands, to trigger the onset of various symptoms. For example, binding of histamine with H1 receptors on the sensory nerve endings quickly leads to sneezing, itching, and excessive mucus secretion. Binding of histamine with either H1 or H2 receptors on the mucosal blood vessels causes the vessels to swell, eventually leading to congestion. Nasal blockage and excessive discharge may also result from the binding between leukotriene and CysLT1 or CysLT2 receptors on blood vessels and secretary glands in the nasal cavity. Meanwhile, these mediators attract more mast cells and basophils, causing the release of more mediators. This self-driving vicious cycle causes the symptoms to become more and more severe. Chronic inflammation, the third stage of the development of allergic responses, is characterized by the hyper-responsiveness of the nose to allergens and non-allergic stimuli. At this stage, more and different types of inflammatory cells are drawn to the sites of inflammation and become activated. These cells include, but are not limited to, basophils, eosinophils, neutrophils, monocytes, and various T cells. The chemokines and cytokines released not only attract more inflammatory cells, but also sustain the existing

inflammation, resulting in the gradual damage of the nasal epithelial cells, and consequently increase the permeability of the nasal epithelium. Therefore, the allergens can more easily penetrate the epithelium barrier, encounter IgE bearing cells, and trigger the following inflammatory reactions. Moreover, the prolonged inflammation makes the nerve endings, mucosal blood vessels, and seromucous glands more sensitive, accounting for their heightened response to allergens and stimuli that are not allergen in nature, for instance, cold air and smoke[1,4].

The current treatment for AR includes antihistamine medication, nasal corticosteroid sprays, nasal antihistamine sprays, and subcutaneous injection of allergens. While being able to reduce the discomfort level of AR effectively, each therapy has its own side effects, with dry mouth and drowsiness being the most common. Additionally, AR brings a heavy financial burden. In 2002, it was reported that every AR patient spent on average of about $300 on physician office visits and medication. There is also indirect financial loss associated with absence from work[5]. Therefore, it is important to discover new treatments that decrease or eliminate side effects, and are less expensive. The use of probiotics has shown such promise.

Etymologically, probiotics means “for life”[6]. The concept of probiotics was first proposed in 1907 by Eli Metchnikoff, who won the Nobel Prize for his pioneering work in the field of intestinal microecology. He stated that useful microbes and harmful microbes coexisted in the human intestinal flora, and that it was probable to alter the flora’s composition[7]. In 1965, Lilly and Still coined the term probiotics, defining substances produced by micro-organisms that had the capability to promote the growth of other micro-organisms[8]. Currently, a widely accepted definition of probiotics is the one from WHO: “live organisms when administered in adequate amounts confer a health benefit on the host”[7]. The rationale of using probiotics in

treating AR lies in the discoveries that 1) the GI tract, the largest immune organ, plays a significant role in the regulation of immune function; 2) there are considerable differences in the composition of the intestinal flora between AR patients and healthy individuals; and 3) probiotics are able to change the composition of intestinal flora very quickly[9].

The intestinal epithelium is the largest immune organ of the body with multiple layers comprising its defensive system[6]. The first layer is a physical barrier between the environment and the body. This barrier is formed by the tight junctions and adhesion junctions along the lateral membranes of the epithelial cells. With fibrils made of complex lipoproteins, permeability of these junctions can be regulated. For instance, pro-inflammatory cytokines like TNF-α can induce the internalization of these junctions, thus disrupting the barrier. The second layer is an antibacterial barrier established by epithelial cells and phagocytes that reside in the intestine due to their ability to produce antimicrobial peptides. These peptides identify microbes then insert themselves into their cell membranes, leading to the rupture and death of the microbes. Additionally, these peptides can interfere with the metabolic and biosynthetic pathways of the pathogens. The third line of defense is the intestinal flora, which is composed of over 400 species with a cell count of approximately 10^{14}. Healthy intestine flora produce organic acids and hydrogen sulfide, forming a hostile environment to reduce the colonization of pathogens. Moreover, the flora constantly and actively interact with the epithelial cells to regulate their expression of antimicrobial peptides[6].

The barrier functions of the intestinal epithelium are reinforced by the body's immune system. Gut-associated lymphoid tissue (GALT) is the largest lymphoid tissue of the body. It is located throughout the intestine and is composed of Peyer's patches, mesenteric lymph nodes, and scattered lymphocytes. Cells in GALT include dendritic cells, macrophages, B cells and T

cells. These immune cells possess the capability to differentiate between self and pathogens primarily through the detection of microbe-associated molecular patterns (MAMPs) on pathogens, such as Toll-like receptors (TLRs) and nucleotide-binding oligomerization domain-like receptors (NLRs). Once pathogens are recognized, the synthesis and secretion of pro-inflammatory cytokines, adhesion molecules, and antimicrobial peptides in these cells and adjacent cells are triggered, thus activating the immune defense system[6].

It is hypothesized that through binding with TLRs, especially TLR-2 and TLR-9, probiotics may initiate specific signal pathways to exert immune regulatory functions[10]. Probiotics might help strengthen the junctions between epithelial cells so that the physical barrier is less permeable to the contents such as food particles and pathogens, in the intestinal lumen. As a result, the body is less likely to mount an abnormal immunological response[10]. Additionally, damaged epithelium heals faster in the presence of probiotics[10]. Probiotics have also been found to decrease the synthesis of pro-inflammatory cytokines such as IL-4, while increasing the level of anti-inflammatory cytokines like IL-10 and IFN-γ. Probiotics are capable of suppressing the IgE production by B cells, which might slow down the progression of AR from the onset[10]. Lastly, probiotics have been reported to divert the body's immune response to a tolerant mode. In two in vitro studies, precursor DCs were primed to transform to DC-1 cells when cultivated with probiotics[1,4]. Since DC-1 cells direct Th0 cells to develop into Th-1 cells, the cytokine profile would be anti-inflammatory[1,4].

In contrast to traditional understanding, the dysbiosis theory, a relatively new theory, proposes that many diseases including autoimmune diseases, allergies, and inflammatory bowel diseases result from the imbalance of microbes in the intestine flora[9]. This theory is supported by the finding that the cell counts of *Lactobacillus spp.* and *Bifidobacterium adolescentis* of AR

patients are much lower than the ones of healthy individuals. The composition of intestine flora is greatly influenced by diet, health status, and environmental factors. The encouraging news is that microbiota composition can be altered within 24 hours after a positive dietary change[9]. All these findings strongly support the practice of dietary intervention in AR patients. It is expected that healthy balanced intestine flora established by direct administration of probiotics, would help the body react to inhaling allergens more mildly, thus limiting the damage caused by inflammation. The objective of this study is to investigate if oral probiotics consumption reduces the allergic symptoms in users compared to non-users.

Chapter II

Review of literature

Two most commonly used bacterial genera as probiotics are *Lactobacillus* and *Bifidobacterium*, both of which are Gram-positive, produce lactic acid, and make up a large fraction of the normal intestinal flora in humans[11]. Members of *Lactobacillus* being investigated for potential probiotic functions include *Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus rhamnosus, Lactobacillus plantarum, Lactobacillus spp., Lactobacillus reuteri, Lactobacillus delbrueckii,*and *Lactobacillus fermentum*. Members of Bifidobacterium being studied consist of *Bifidobacterium spp., Bifidobacterium longum, Bifidobacterium bifidum, Bifidobacterium breve, Bifidobacterium lactis,*and *Bifidobacterium infantis*[11]. A handful of studies also explored the prospectively probiotics roles of other microbes; e.g., yeasts and some *E. coli* strains that are not pathogenic[11]. Probiotics come from two major sources: natural source and nutrition supplement. The natural source refers to different kinds of fermented foods, such as yogurt, kefir, miso, tempeh, and sauerkraut. Probiotics supplements are packaged in various forms: powder, capsule, and even in herbal tea. They have evolved into a multi-billion-dollar market globally with a sale of $1.3 billion in 2010 and is predicted to reach $2.07 billion by 2015[12]. The use of probiotics has been evaluated in several diseases characterized by abnormal immune response. Isolauri et al. reported that the oral administration of *Bifidobacterium* or *Lactobacillus* strains helped relieve the symptoms of food allergy[13]. Anaphylactic symptoms as a result of food allergy were reduced when the probiotics mixture VSL#3 (highly potent probiotic mixture comprising of 8 strains of live bacteria with up to 900 billion counts in one packet[14]) and

Lactobacillus casei strain *Shirota* were consumed[15]. The use of probiotics have been shown to prevent the onset of eczema[16–18]. Probiotics used in these studies include *Lactobacillus rhamnosus, Lactobacillus GG, Lactobacillus reuteri* and *Bifidobacterium lactis*. The prevalence of eczema in the control group was two times higher than that of the intervention group[16,17]. *Lactobacillus GG, Lactobacillus plantarum,* and *Bifidobacterium-12* exerted protective effects in animal models with asthma. The probiotics reduced the allergic inflammation of the animals' airways[19,20], and suppressed the allergen-induced degranulation of basophils. Similar to the above diseases, AR results from abnormal allergic response. To date researchers around the globe have conducted many studies to investigate the effects of probiotics consumption on patients suffering from AR. However, these studies reported inconsistent results. For example, a couple studies reported that the use of probiotics reduced the inflammation in the patients measured by biomarkers including IgE, eosinophils, and IFN-γ, while some studies reported the opposite[21–29]. Several studies showed that probiotics improved the subjective symptoms such as nose symptom and eye symptom of the patients assessed by subjective questionnaires, while some studies concluded that probiotics consumption did not bring about more benefits than placebo[21–29]. These details are presented in the following result section. Furthermore, most of these studies had comparatively small sample size, which limits their capability to draw convincing conclusions. Consequently, the objective of this study is to assess the effectiveness of probiotics consumption in treating AR through meta-analysis, and make suggestions for future studies.

Chapter III

Methods

Inclusion criteria

A study needed to fulfill the following requirements to be included in this analysis: double blinded, placebo-controlled trial; the participants of the studies were adults (>18 years old); the participants had been clinically diagnosed with AR; and reported outcomes included either subjective symptom scores and/or blood biomarker levels of IgE, eosinophils, and IFN-γ.

Search strategy

Three main databases in the field of life science (PubMed, Cochrane and CINAHL), were searched to identify the studies to be included in the meta-analysis. The search was conducted on February 12, 2013, as a result, it is guaranteed that even the most recently published studies are included. Searching PubMed using MeSH words “Rhinitis, Allergic, Seasonal” and “Probiotics” generated 27 hits. Searching Cochrane with “allergic rhinitis” and “probiotics” as “ALL TEXT” found 37 papers. Lastly, using “CINAHL heading”, equivalent to MeSH in PubMed, the search identified 5 articles.

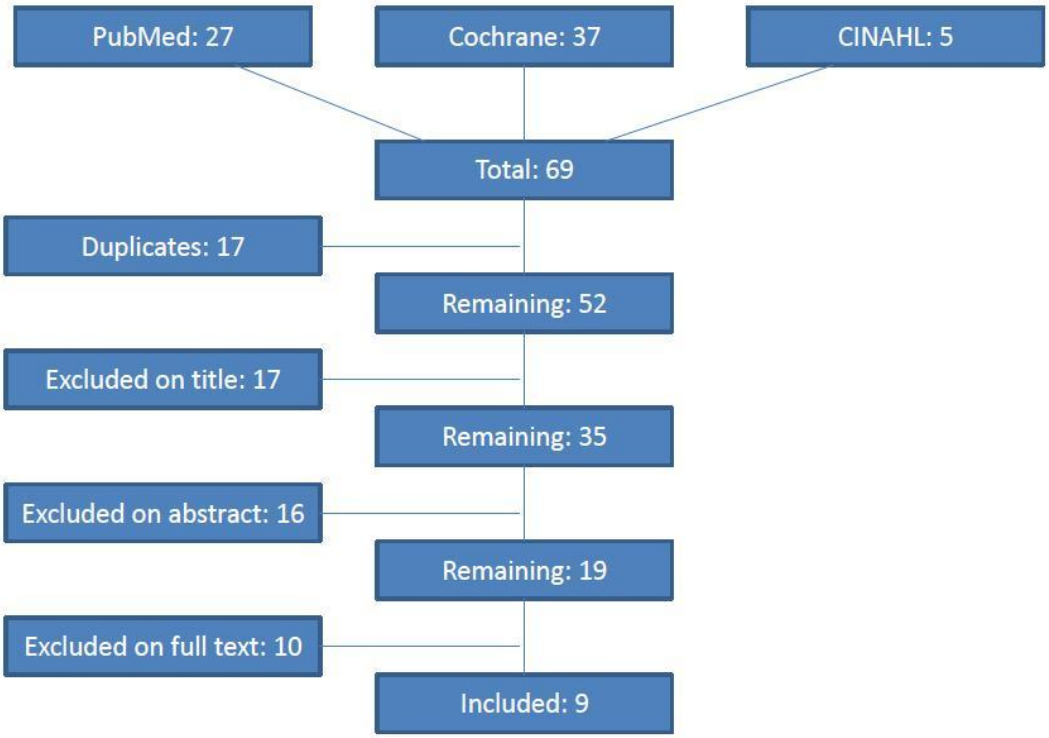

Fig. 1 Flow chart of paper selection

The process of selecting the studies to be included in the meta-analysis is shown in Figure 1. Out of the total 69 papers, 17 papers were excluded as a result of duplication. Seventeen more papers were excluded based on their title, primarily because the study used animal models, or the participants were not adults. Another reason for the exclusion of some studies based on title is that they were published in a foreign language that could not be easily understood. Sixteen papers were further excluded based on the abstract since they did not meet the inclusion criteria; e.g., the experiment was conducted on children or infants, or the study reported outcomes other than subjective symptoms or blood biomarker levels like fecal microbe composition. Lastly, after reading through the whole text, 10 additional papers were excluded due to the fact that when reporting the outcomes of interest, they did not present data in forms that could be used in the meta-analysis. For example, some studies presented the results as

figures, but did not report the actual values, such as mean and SD; other studies showed the results as ratios of post-intervention over pre-intervention measurement; and some studies simply made a statement of not finding significant difference between the intervention group and the placebo group, while giving no detailed values.

The general information of the 9 selected studies[21–29], such as the year of the publication, the duration of the intervention, the strain of probiotics that is used, the number of participants, and the synopsis of the results, is summarized in Appendix 1.

Data extraction

The following information is extracted from the selected studies for the analysis: first author and publication year, duration of the study, each outcome with respective pre- and post-measurement of means and standard deviations for control and intervention group, sample sizes, the dosage of the intervention, and the form of the intervention.

Statistical analysis

Comprehensive Meta-Analysis Version 2.0, commercial meta-analysis software, was used to perform the statistical analysis. Because different methods were used to measure outcomes in the selected studies, standardized mean difference was chosen as the effect size of individual studies. Randomized effects model was used to calculate the overall effect size, because of the existence of several differences among the studies: probiotic strains, varied duration of the intervention, and inherent genetic difference of the participants. In addition, due to the generally small sample sizes, Hedges' g, standardized mean difference after correction, was used for further analysis and plotting. Corresponding 95% CIs were calculated. Lastly, meta-analyses of the comparison between the placebo and probiotic groups were conducted on the following outcomes: allergen-specific IgE, eosinophil count, IFN-γ, Th1/Th2, eye symptom

score, nose symptom score, and overall symptom score. In studies that measured similar outcomes, the values of measured outcomes were combined to determine the effect size. For example, sneezing, runny nose, stuffy nose, and itchy nose were grouped together as nose symptoms. The study by Koyama in which IgE against three different types of pollens were measured, their values were combined during the analysis due to the similar nature of the three measures.

Two subgroup analyses were then performed. One assessed if there was a difference in the effects on symptoms vs. blood biomarker between placebo and probiotic groups. The other one assessed if there were different effects between the uses of dairy vs. non-dairy as the treatment.

Chapter IV

Results

IgE

Seven selected studies[21–26,29] reported IgE as an outcome, and none of them presented a statistically significant difference between the placebo and the probiotics group. The overall effect barely favors the placebo group, with the overall effect size of -0.085 (Fig. 2), but is not statistically significant. (p=0.468, Appendix 2).

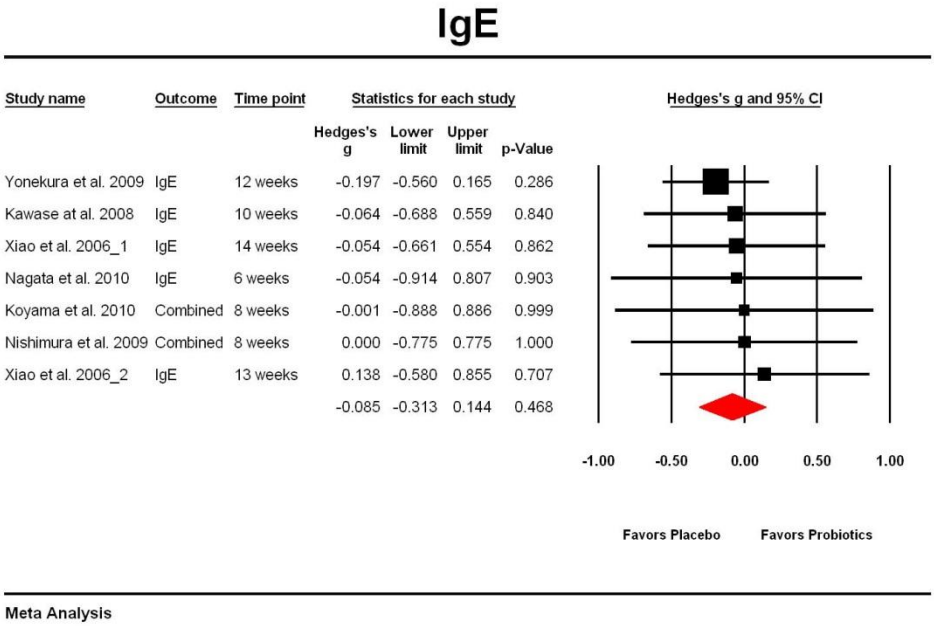

Fig. 2 Meta-analysis based on the outcome of IgE

Eosinophil

Five selected studies[24–27,29] reported either eosinophil count or eosinophil % as an outcome, and only one of the studies[25] found a statistically significant difference between the placebo and the probiotics group, with an effect size of -0.801, and a p value of 0.016 (Fig. 3). The overall effect slightly favors the placebo group, with the overall effect size of -0.145 (Fig. 3), but is not statistically significant. (p=0.371, Appendix 3).

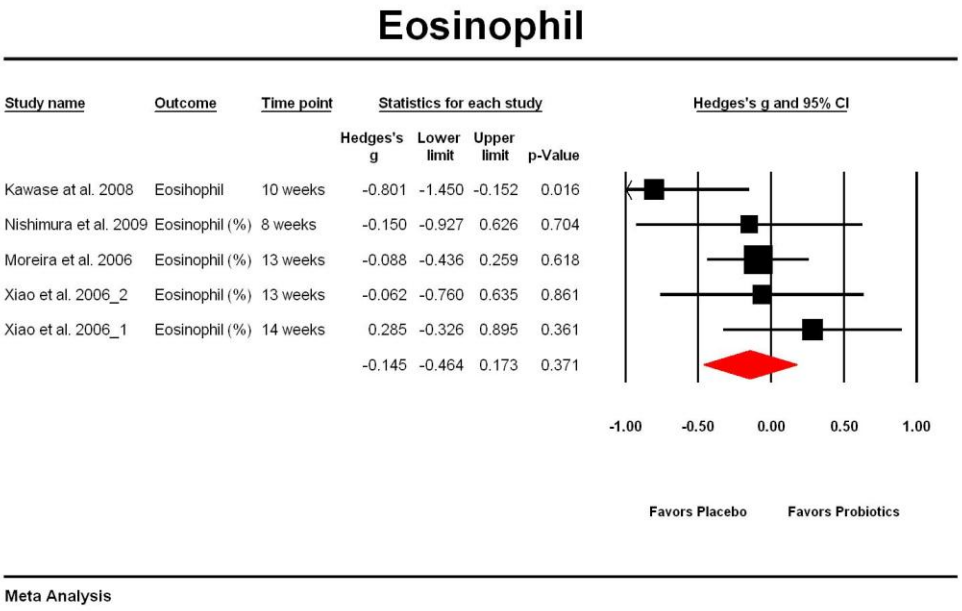

Fig. 3 Meta-analysis based on the outcome of eosinophil

IFN-γ

Three selected studies[21,24,26] reported IFN-γ as an outcome with one[24] presenting a statistically significant difference between the placebo and the probiotics group, with an effect size of -1.037, and a p value of 0.006 (Fig. 4). The overall effect moderately favors the placebo group, with the overall effect size of -0.529 (Fig. 4), which is almost statistically significant (p=0.067, Appendix 4).

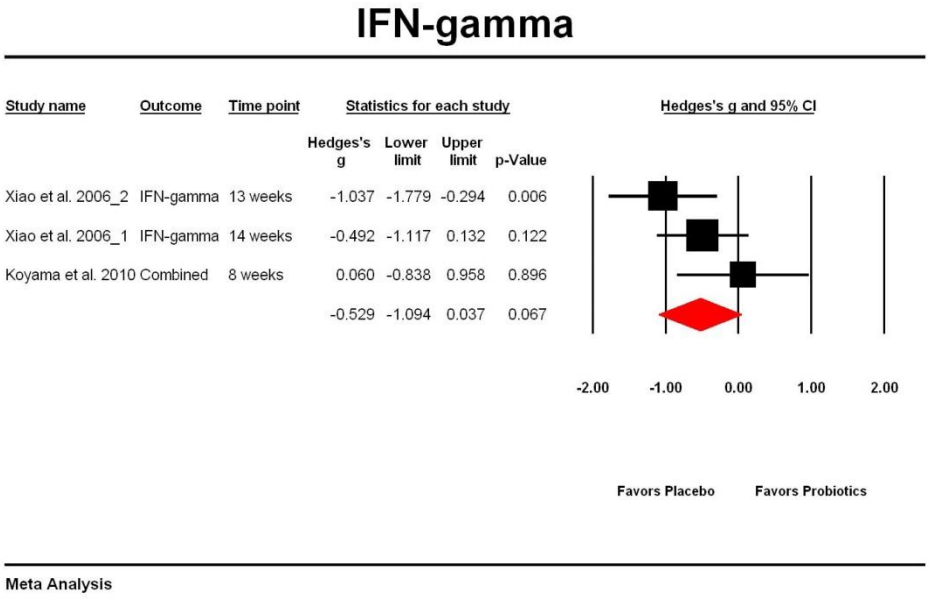

Fig. 4 Meta-analysis based on the outcome of IFN-γ

Th1/Th2

Three selected studies[22,23,25] reported Th1/Th2 as an outcome with one[25] presenting a statistically significant difference between the placebo and the probiotics group, with an effect size of -1.865, and a p value of 0.000 (Fig. 5). The overall effect moderately favors the placebo group, with the overall effect size of -0.535 (Fig. 5), but is not statistically significant (p=0.391, Appendix 5).

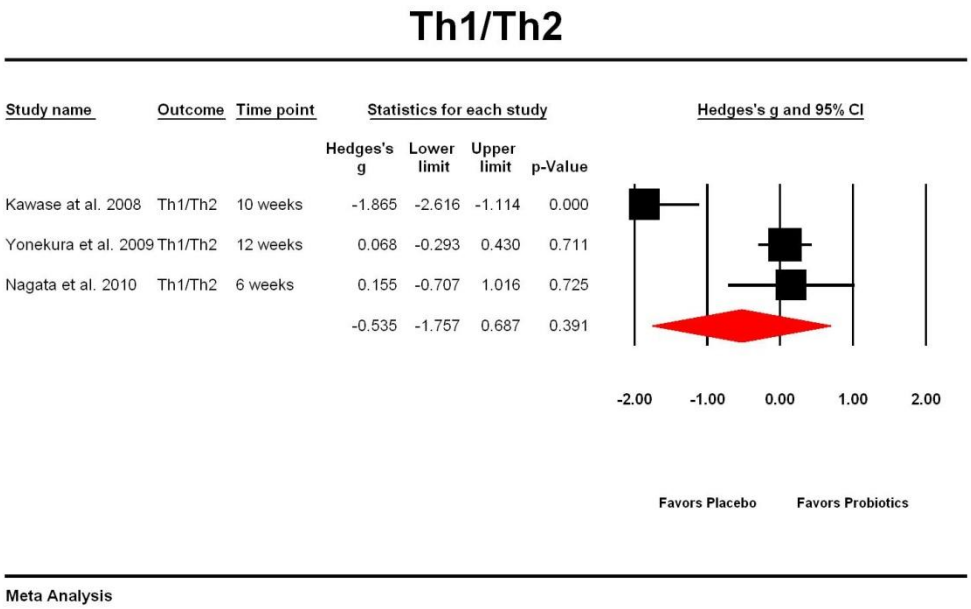

Fig. 5 Meta-analysis based on the outcome of Th1/Th2

Eye symptoms

Three selected studies[23,24,26] reported eye-related symptom scores as outcomes, with one[24] presenting a statistically significant difference between the placebo and the probiotics group, with an effect size of 0.594, and a p value of 0.05 (Fig. 6). The overall effect moderately favors the probiotic group, with the overall effect size of 0.35 (Fig. 6), and is statistically significant (p=0.013, Appendix 6).

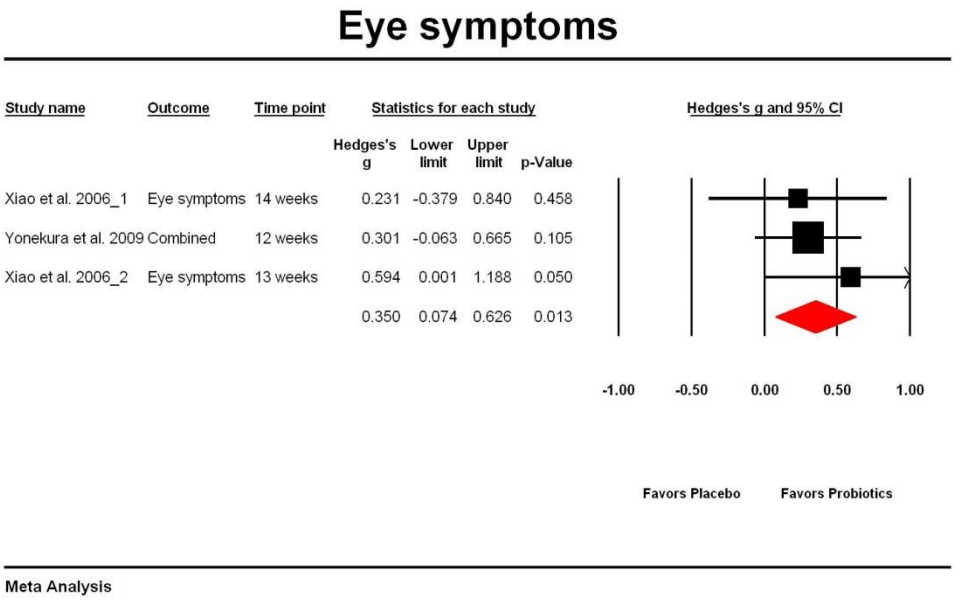

Fig. 6 Meta-analysis based on the outcome of subjective eye symptoms score

Nose symptoms

The same three studies[23,24,26] reported nose-related symptom scores as outcomes, with one[24] presenting a statistically significant difference between the placebo and the probiotic group, with an effect size of 0.781, and a p value of 0.011 (Fig. 7). The overall effect moderately favors the probiotic group, with the overall effect size of 0.349 (Fig. 7), and is barely statistically significant (p=0.047, Appendix 7).

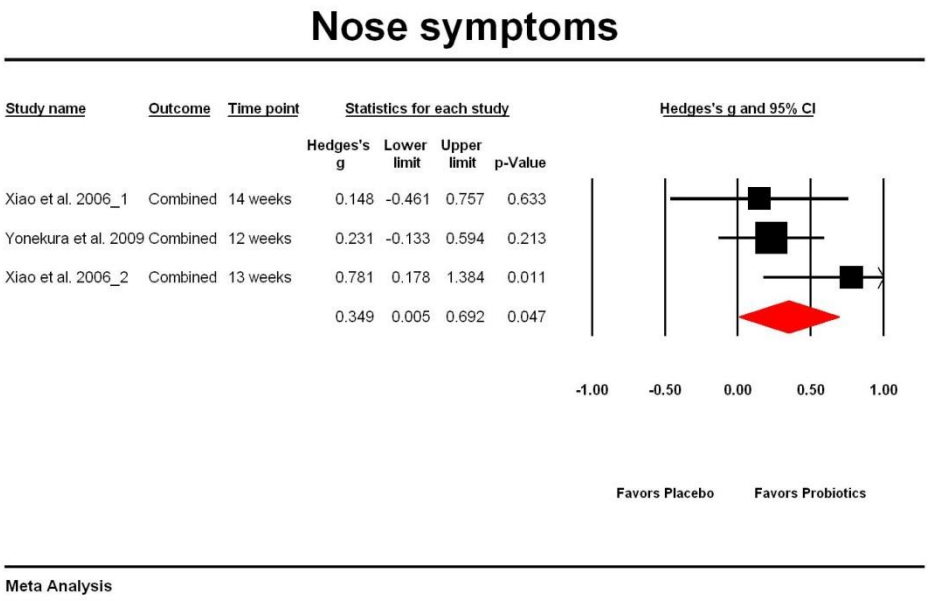

Fig. 7 Meta-analysis based on the outcome of subjective nose symptoms score

Overall subjective symptom scores

Four selected studies[23,24,26,28] reported subjective symptom scores as outcomes with two[24,28] presenting a statistically significant difference between the placebo and the probiotics group, with an effect sizes of 0.730 and 1.315, and a p values of 0.017 and 0.023, respectively (Fig. 8). The overall effect moderately favors the probiotic group, with the overall effect size of 0.451 (Fig. 8), and it is statistically significant (p=0.020, Appendix 8).

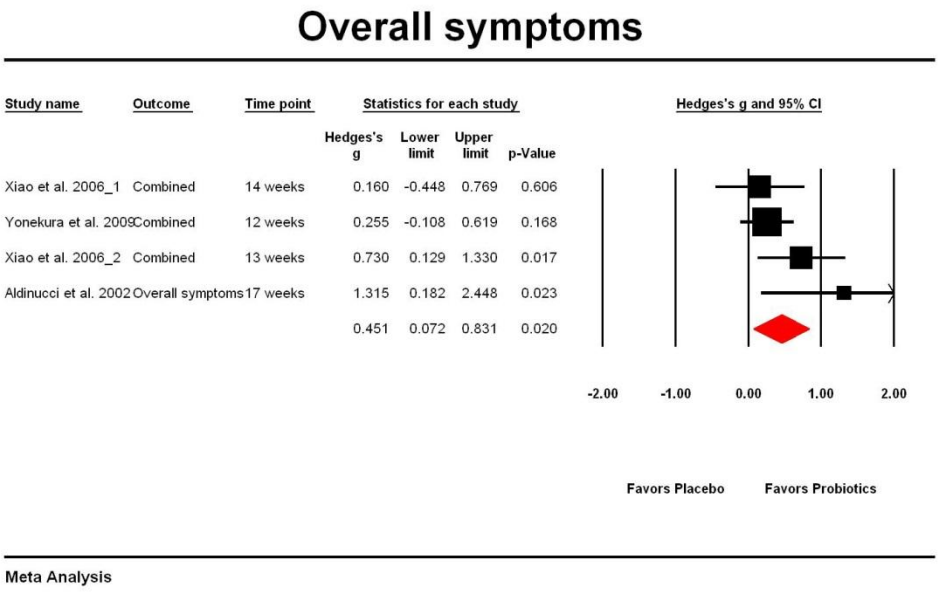

Fig. 8 Meta-analysis based on the outcome of overall subjective symptoms score

Blood biomarkers vs. symptom scores

Subgroup analysis based on blood biomarkers vs. symptom scores reveals that the placebo appeared to have induced better outcomes with regard to the measurement of blood biomarkers, but this seemly superior effect is not statistically significant, with an overall effect size of -0.150, and a p value of 0.127 (Fig. 9). Judging based on the subjective symptom scores, probiotics show a statistically significantly better outcome than the placebo, with an overall effect size of 0.451, and a p value of 0.02 (Fig. 9). And the difference between the two subgroup analysis is statistically significant (p=0.006, Appendix 9).

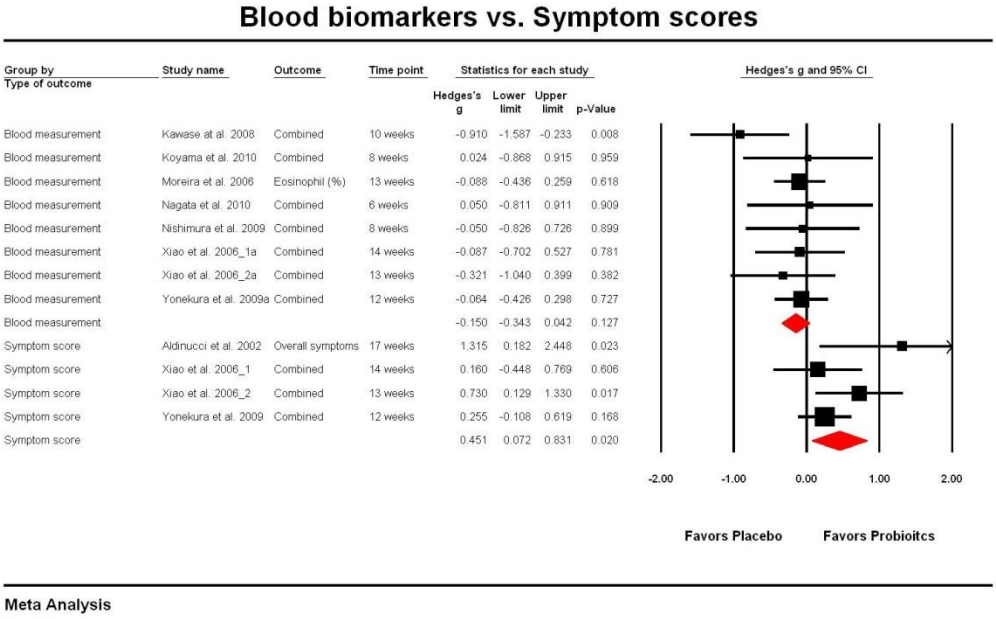

Fig. 9 Subgroup analysis based on the outcome of blood biomarkers measurement vs. subjective symptoms score

Dairy vs. Non-dairy

Subgroup analysis based on the type of the intervention, dairy form vs. non-dairy form of the probiotics, reveals that intervention with non-dairy form seems to have produced better overall outcomes. The outcomes were calculated combining both blood biomarkers measurement and subjective symptoms score. The superior effect of non-dairy intervention is not statistically significant, with an overall effect size of 0.184, and p value of 0.200 (Fig. 10). Intervention with dairy form actually had a worse performance than the placebo group, with an overall effect size of -0.030, and a p value of 0.912 (Fig. 10). The difference between the two subgroup analysis is not statistically significant (p=0.2, Appendix 10).

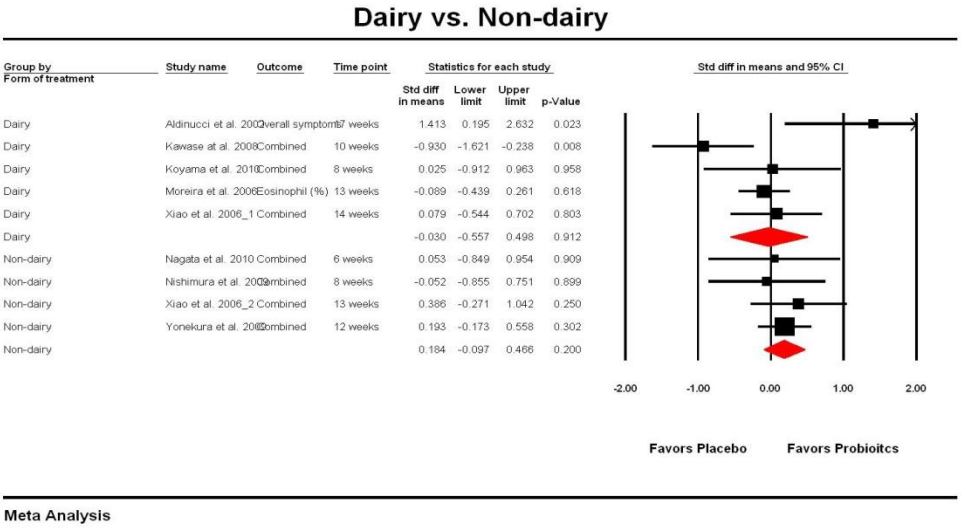

Fig. 10 Subgroup analysis based on the type of the intervention: dairy form vs. non-dairy form

Chapter V

Discussion and Conclusions

The results of the meta-analysis in this study showed that the participants from the groups consuming probiotics reported an overall lower score for subjective symptoms, compared to those from the placebo groups, and the difference between the two groups was statistically significant with a p value of 0.02 (Fig. 9). In other words, probiotics consumption indeed effectively reduced the allergic symptoms. However, the observed improvement of the subjective symptoms scores did not align with the objective measurement of the concentration of the serum biomarkers. As a matter of fact, the results of all the objective measurements indicated an inferior effect of the probiotics treatment than the placebo treatment, although none reached a statistically significant level. Many reasons could potentially explain this contradiction. First of all, the researchers might not have chosen reliable serum biomarkers. Second, even if a good choice of biomarkers was made, the researchers might not have collected the most optimal sample for measurement. Lastly, there reason has been debate over the merits of subjective symptoms assessment, since widespread doubt exists in the life science field regarding the assessment of subjective symptoms through questionnaires.

Theoretically, every molecule involved in the allergic chain reaction could be a biomarker for the assessment of the AR status, but due to the varied biological attributes of these molecules and the technical difficulties of the measurement, only a few have been used as biomarkers in AR-related research studies. Even fewer of them have been evaluated for their

validity and reproducibility. Levels of IgE, eosinophil count, Th1/Th2 ratio, and IFN-γ concentration are parameters that have drawn the most attention.

IgE

The majority of the included studies (7 out of 9) reported IgE level as an outcome. Unfortunately, most of these studies simply stated that the peripheral serum sample was collected for the measurement of IgE level, while providing no further details regarding how the measurement was taken. The remaining studies failed to mention the type of sample that was collected for the subsequent measurement. After being captured by mast cells and basophils through FcεRI receptors on their cell membrane, IgEs crosslink in the presence of specific allergens, causing the degranulation of the effector cell to which they bind, which is then followed by the progression of the allergic responses[30]. Based on the understanding of this vital role of IgE in the development of AR, drugs capable of interfering with the interaction between IgE and its effector cells have been designed to treat allergic diseases. Omalizumab, humanized antibody against IgE, is an example. Omalizumab reduces the symptoms of allergic asthma, and decreases the use of other traditional medications[31]. Therefore, IgE level possesses the attributes of a reliable biomarker, both in theory and in practice. However, two questions need to be answered regarding its use for assessing the severity of the AR. First, since IgE exists in the body in two forms, cell-bound and free form, it is important to find out which one is the better marker. Second, IgEs can be found in the blood circulation as well as in the nasal fluid discharge; therefore, it is equally important to know which fraction of IgE is more relevant when studying AR. The majority of IgEs tightly bind to the cell membrane of mast cells and basophils, due to this physical connection, these IgEs exist for a long period of time. On the other hand, the freely floating IgEs in blood circulation are not stable, having a half-life of about two days[31]. Therefore,

support for choosing free-form IgE over cell-bound IgE as the biomarker seems reasonable, because studies of AR are generally constrained by the length of the pollen season, which lasts two to three months. This might not leave enough time for a molecule with a long life-time to show a change. In contrast, measuring a molecule with a quick turn-over rate assesses the real-time response of the body, in this case, IgE synthesis to the intervention of probiotics consumption. Using free-form IgEs as the biomarker has been vindicated by the finding from studies using flow cytometry: free IgEs in the serum sample positively correlate with the concentration of IgE-bearing monocytes and basophils[31]. Additionally, the technique of measuring free IgEs in serum sample is considerably easier than measuring IgEs bound to the effector cells.

IgE-producing cells and IgEs have been easily detected in the nasal lavage fluid that is collected by simple techniques such as Nasal lavage (NAL) technique. In comparison, nasal brushes (NAB), which obtains cells from the nasal mucosa, is more accurate at evaluating the cell profile[31]. Plasma cells (PCs), one form of activated B cells that secret specific IgEs, reside not only in the bone marrow but also in the respiratory mucosa[31]. During the pollen season, the majority of the IgEs that are grass-pollen specific are secreted by the PCs in the respiratory mucosa; and this de novo synthesis and secretion of pollen-specific IgEs take place throughout the whole pollen season[31]. Because of the close connection between IgEs with mast cells and basophils, measuring IgE levels at where symptoms occur, e.g. nose, should be more relevant and more accurate at indicating the severity of the inflammation.

Eosinophils

Most of the included studies (7 out of 9) reported either eosinophil count or eosinophil % as an outcome. In six of these seven studies, eosinophils from serum samples were counted. One

study counted eosinophils from the nasal lavage fluid samples. None of the authors provided details regarding how the counting was conducted. Activated eosinophil is a key effector cell type that plays important role in maintaining the inflammation of the nasal mucosa, thus leading to a chronic inflammatory status of the nose. Therefore, eosinophil count should be a very informative biomarker for evaluating the severity of the nasal chronic inflammation in patients with AR. However, it is crucial to distinguish activated eosinophils from non-activated eosinophils. A major difference between the two is that only activated eosinophils synthesize and present BMK13 on their cell membranes[32]. Researchers have also begun using eosinophil cation protein (ECP) as a surrogate for activated eosinophils, because ECP is a soluble protein only secreted by activated eosinophils. Along with the transition from non-pollen season to pollen season, the level of ECP in AR patients increases dramatically. Additionally, a moderate correlation (r=0.53) has been reported between ECP concentration and nasal symptoms during the pollen season[32]. Furthermore, ECP has been measured in drug trials to evaluate the effectiveness of the treatment. In one study, the application of fluticasone, a widely used nasal corticosteroids spray, reduced the average ECP level of AR patients to one sixth of its starting value. Additionally, the severity of allergic symptoms assessed by subjective symptom questionnaires decreased by 75%[32]. Considering that measuring the concentration of a soluble substance generally has much lower requirement for instruments and skills than measuring the quantity of a certain type of cell with specific surface molecules, ECP appears to be a better option.

Both eosinophils and ECP can be easily measured from the nasal lavage fluid. Since eosinophils are recruited to the site of inflammation (nose in the case of AR) and function to maintain the inflammatory status, the measurement at the local level, compared to the

measurement in the blood, should be more sensitive at detecting the change of the severity of the allergic symptoms caused by the intervention.

IFN-γ

A small number of the selected studies (3 out of 9) reported the measurement of IFN-γ level from serum sample as an outcome. IFN-γ is a Th-1 specific cytokine[30] that is capable of inhibiting the transformation of Th0 to Th2 cells. Additionally, IFN-γ can suppress the inflammatory reactions of the effector cells, including mast cells and eosinophils[33]. Although many researchers conducting studies related to inflammation have measured IFN-γ level, its applicability as a biomarkers for studies about AR has not been established. The underlying reason might be the contradictory functions of IFN-γ. While possessing the ability to suppress the allergic reactions, IFN-γ is a strong pro-inflammatory cytokine and a vital signal for the activation of macrophages. Activated macrophages release highly toxic molecules, such as oxygen radicals and nitric oxide, causing localized tissue damage[30]. Neither high nor low levels of IFN-γ can indicate an improved inflammatory status of the local organ or the whole body, so alone it would not be a good choice as a biomarker.

Th1/Th2

A small number of the selected studies (3 out of 9) reported the ratio of Th1/Th2 from serum sample as an outcome. One study provided no details regarding the test, and the other two reported counting cells using flow cytometry. Th1 cells were identified with CD4+, IFN-γ+, and IL4-, while Th2 cells were identified with CD4+, IFN-γ-, and IL-4+. The cytokine profile of Th2 cells is pro-allergenic, primarily because IL-4 promotes the class switching of immunoglobulins in B cells, giving rise to the synthesis of allergen-specific IgE. In contrast, the cytokines produced by Th1 cells, including IFN-γ and IL-12, exert an inhibitory effect on the transformation of Th0 cells into Th2 cells. Decreased Th2 cell count induced by immunotherapy,

and the consequent shift of the balance from Th2 towards Th1 side, was found to be associated with lower subjective symptoms scores and less medication use[33]. Nevertheless, an abnormally skewed balance of Th1/Th2 towards the Th1 side carries its own risk of rendering individuals vulnerable to develop contact dermatitis as the result of macrophage activation. An example of contact dermatitis is the development of rash, sometimes even blisters, after touching poison ivy[30]. Therefore, Th1/Th2 appears to be a good biomarker for studies about AR as long as it is within a certain range. Th1 and Th2 cells play important roles at the early stage of the development of AR, and their ratio seems to set the tone of the overall inflammatory status of the body. Therefore, measuring the ratio is appropriate to assess potential long-term effects. Measuring Th1/Th2 from serum sample also supports the fundamental idea behind the use probiotics for AR management: the human body exists as an integrative entity, so an event taking place at one part of the body has influence on other parts of the body. Generally, such effects occur as a result of certain molecules traveling through the circulatory system. Positively altering the composition of the microbiota in the intestine should first lead to positive changes of immune response locally, then throughout the whole body, as a result improving diseases with abnormal immune response in nature.

Subjective symptoms scores

Researchers in the field of life science generally are reluctant to use subjective perception as the outcome, because these measurements indeed vary significantly between individuals, and they cannot be objectively and repeatedly quantified. These outcomes are easily affected by factors other than the treatment, such as mood change, energy level, and trivial things that happen in everyday life. Probably due to these concerns, less than half of the selected studies that are included in this analysis assessed and reported the subjective symptoms scores. If a study is a double-blinded and placebo-controlled trial, the psychological influence due to placebo effect

should be ruled out; moreover, interpersonal variance and mood changes are unlikely to mask or even reverse the effects of the treatment as long as the sample size is big enough and the duration of the intervention is long enough. Furthermore, drug trials, which have the reputation of being rigorous, have repeatedly reported the improvement in subjective symptoms scores due to the drug application. The intervention with probiotics consumption is conducted on people with AR living in a real world, where confounding factors are difficult to control. What really matters to a patient is how he/she perceives his/her quality of life. Even golden biomarkers are discovered, the assessment of subjective symptoms still has its advantages, because human beings exist as a inseparable entity of subjective body and objective soul.

In summary, the intervention of probiotics consumption has low risk, is cost-effective, and is capable of decreasing symptoms and improving life quality. Therefore, Probiotics are worth trying for AR patients who are open to alternative therapies.

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Appendices

1. Summary of included studies

First author and year of publication	Duration of the study	Total participants	Age range (years)	Country	Strain of probiotics	Form of intervention	Results
Koyama et al. 2010	8 weeks (Spring season)	19	21-54	Canada	*Lactobacillus rhamnosus GR-1* and *Bifidobacterium adolescentis*	Yogurt	Antigen-specific IgE (Spring): ↓ Antigen-specific IgE (Fall): ↑ IFN-γ (Spring and Fall): ↑
	8 weeks (Fall season)	16	21-58				
Nagata et al. 2010	6 weeks (Spring season)	33	21.9 (5.6)	Japan	*Lactobacillus plantarum* No. 14	Powder	Antigen-specific IgE: ↓ Th1/Th2: ↑
Yonekura et al. 2009	12 weeks	116	20-50	Japan	Lactobacillus paracasei KW3110	Powder	Antigen-specific IgE: ↓ Th1/Th2: ↓ Eye symptoms: ↑ Nose symptoms: ↑
Xiao et al. 2006	13 weeks	44	22-57	Japan	*Bifidobacterium longum BBS36*	Powder	Antigen-specific IgE: ↑ Eosinophil: ↓ IFN-γ: ↓ Eye symptoms: ↑ Nose symptoms: ↑ Throat symptoms: ↑

Kawase at al. 2008	10 weeks	38	20-57	Japan	*Lactobacillus GG* and *L.gasseri TMC0356*	Fermented milk	Antigen-specific IgE: ↓ Eosinophil: ↓ Th1/Th2: ↓
Xiao et al. 2006	14 weeks	40	23-61	Japan	*Bifidobacterium longum BBS36*	Yogurt	Antigen-specific IgE: ↓ IFN-γ: ↓ Eosinophil: ↑ Eye symptoms: ↑ Nose symptoms: ↑ Throat symptoms: ↑
Moreira et al. 2006	13 weeks	126	40 (10)	Finland	*Lactobacillus GG*	Fermented milk	Eosinophil: ↓
Aldinucci et al. 2002	17 weeks	13	19-44	Italy	*Lactobacillus acidophylus* and *Bifidobacterium*	Yogurt	Overall subjective symtoms: ↑
Nishimura et al. 2009	8 weeks	24	33.8 (2)	Japan	*Tetragenococcus halophilus* Th221	Tablet	Antigen-specific IgE: ↓ Eosinophil: ↓
Sum		469					

↑: the probiotics group appeared to show better outcome than the placebo group

↓: the probiotics group appeared to show worse outcome than the placebo group

2. Test of statistical significance of IgE level between probiotics groups and placebo groups

Model		Effect size and 95% confidence interval					Test of null (2-Tail)		Heterogeneity		
Model	Number Studies	Point estimate	Standard error	Variance	Lower limit	Upper limit	Z-value	P-value	Q-value	df (Q)	P-value
Fixed	7	-0.085	0.117	0.014	-0.313	0.144	-0.726	0.468	0.839	6	0.991
Random	7	-0.085	0.117	0.014	-0.313	0.144	-0.726	0.468			

3. Test of statistical significance of eosinophil count between probiotics groups and placebo groups

Model		Effect size and 95% confidence interval					Test of null (2-Tail)		Heterogeneity		
Model	Number Studies	Point estimate	Standard error	Variance	Lower limit	Upper limit	Z-value	P-value	Q-value	df (Q)	P-value
Fixed	5	-0.132	0.124	0.015	-0.374	0.110	-1.068	0.286	5.976	4	0.201
Random	5	-0.145	0.162	0.026	-0.464	0.173	-0.895	0.371			

4. Test of statistical significance of IFN-γ level between probiotics groups and placebo groups

Model		Effect size and 95% confidence interval					Test of null (2-Tail)		Heterogeneity		
Model	Number Studies	Point estimate	Standard error	Variance	Lower limit	Upper limit	Z-value	P-value	Q-value	df (Q)	P-value
Fixed	3	-0.546	0.215	0.046	-0.968	-0.124	-2.537	0.011	3.457	2	0.178
Random	3	-0.529	0.289	0.083	-1.094	0.037	-1.831	0.067			

5. Test of statistical significance of Th1/Th2 between probiotics groups and placebo groups

Model		Effect size and 95% confidence interval					Test of null (2-Tail)		Heterogeneity		
Model	Number Studies	Point estimate	Standard error	Variance	Lower limit	Upper limit	Z-value	P-value	Q-value	df (Q)	P-value
Fixed	3	-0.239	0.156	0.024	-0.544	0.066	-1.537	0.124	21.573	2	0.000
Random	3	-0.535	0.624	0.389	-1.757	0.687	-0.858	0.391			

6. Test of statistical significance of eye symptoms score between probiotics groups and placebo groups

Model		Effect size and 95% confidence interval					Test of null (2-Tail)		Heterogeneity		
Model	Number Studies	Point estimate	Standard error	Variance	Lower limit	Upper limit	Z-value	P-value	Q-value	df (Q)	P-value
Fixed	3	0.350	0.141	0.020	0.074	0.626	2.481	0.013	0.867	2	0.648
Random	3	0.350	0.141	0.020	0.074	0.626	2.481	0.013			

7. Test of statistical significance of nose symptoms score between probiotics groups and placebo groups

Model		Effect size and 95% confidence interval					Test of null (2-Tail)		Heterogeneity		
Model	Number Studies	Point estimate	Standard error	Variance	Lower limit	Upper limit	Z-value	P-value	Q-value	df (Q)	P-value
Fixed	3	0.330	0.141	0.020	0.053	0.607	2.332	0.020	2.781	2	0.249
Random	3	0.349	0.175	0.031	0.005	0.692	1.991	0.047			

8. Test of statistical significance of overall objective symptoms score between probiotics groups and placebo groups

Model		Effect size and 95% confidence interval					Test of null (2-Tail)		Heterogeneity		
Model	Number Studies	Point estimate	Standard error	Variance	Lower limit	Upper limit	Z-value	P-value	Q-value	df (Q)	P-value
Fixed	4	0.392	0.137	0.019	0.123	0.661	2.854	0.004	4.862	3	0.182
Random	4	0.451	0.193	0.037	0.072	0.831	2.333	0.020			

9. Test of statistical significance of subgroup analysis of blood biomarkers level vs. symptoms score

Groups		Effect size and 95% confidence interval					Test of null (2-Tail)		Heterogeneity		
Group	Number Studies	Point estimate	Standard error	Variance	Lower limit	Upper limit	Z-value	P-value	Q-value	df (Q)	P-value
Fixed effect analysis											
Blood	8	-0.150	0.098	0.010	-0.343	0.042	-1.528	0.127	5.854	7	0.557
Symptom score	4	0.392	0.137	0.019	0.123	0.661	2.854	0.004	4.862	3	0.182
Total within									10.716	10	0.380
Total between									10.302	1	0.001
Random effects analysis											
Blood	8	-0.150	0.098	0.010	-0.343	0.042	-1.528	0.127			
Symptom score	4	0.451	0.193	0.037	0.072	0.831	2.333	0.020			
Total between									7.686	1	0.006

10. Test of statistical significance of subgroup analysis of dairy-form intervention vs. non-dairy-form intervention

Groups		Effect size and 95% confidence interval					Test of null (2-Tail)		Heterogeneity		
Group	Number Studies	Point estimate	Standard error	Variance	Lower limit	Upper limit	Z-value	P-value	Q-value	df (Q)	P-value
Fixed effect analysis											
Dairy	5	-0.101	0.133	0.018	-0.363	0.160	-0.761	0.447	11.851	4	0.018
Non-dairy	4	0.184	0.144	0.021	-0.097	0.466	1.283	0.200	0.777	3	0.855
Total within									12.629	7	0.082
Total between									2.126	1	0.145
Random effects analysis											
Dairy	5	-0.030	0.269	0.072	-0.557	0.498	-0.111	0.912			
Non-dairy	4	0.184	0.144	0.021	-0.097	0.466	1.283	0.200			
Total between									0.493	1	0.483